

# Supporting Information

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## SI Text

**$\beta$ -Galactosidase Staining of VNO Sections.**  $\beta$ -Galactosidase stains of VNOs from wild-type C57BL6/J mice were performed as previously described for visualizing endogenous  $\beta$ -galactosidase activity in the olfactory epithelium (1).

## Fpr-Specific Primer Pairs

### Fpr1

- 1- CATGTCTCTCCTCATGAACAAG
- 2- ATGAGAAGACATCCAGAACGA

### Fpr-rs1

- 1- CCTTCCCGAGTTCTTACAGG
- 2- CACTAAACTGCATCTCTTTGAG

### Fpr-rs2

- 1- TGACTACTGTTAGAATTCCTG
- 2- GATAGCTGTGTTCAACTTTTCTTCAT

### Fpr-rs3

- 1- CAAATTTGTTCTCAGCATTGTGC
- 2- TACTGGGTGCAGGACACAAG

### Fpr-rs4

- 1- AGGGGATGTGACTGTATATC
- 2- GATTCTGAAGCTGTGGCAG

### Fpr-rs6

- 1- GAAAGTGGGAAAGTACATTGC
- 2- CTCACAGTCATAGATACTTTTACT

### Fpr-rs7

- 1- TTCTGATGCTTATATTTCCACATTTTTT
- 2- CCCAGGATTCAAATTGCATATACAG

**BLAST Search Criteria.** Additional *Fpr* gene sequences, listed below, were retrieved from genome databases at NCBI using TBLASTN. Sequences encoding proteins with at least 45% identity to any mouse FPR are described as FPRs. Six platypus, three opossum, and one zebrafish sequences share  $\approx 40\%$  identity with FPRs, but it is not clear that these are FPRs because they also share  $\approx 40\%$  identity with other GPCRs and appeared to arise from independent gene expansions from those that gave rise to FPRs in placental mammals. A partial *Fpr* gene (from cat) and *Fpr* pseudogenes (from mouse and horse) were not included in the analysis.

**Phylogenetic Analysis.** Protein sequences encoded by *Fpr* genes and outgroup sequences were aligned using the MUSCLE algorithm (2), and a phylogenetic tree was built using MrBayes (3) with the mixed model for amino acid substitutions. MrBayes was run for 3,000,000 generations with  $2 \times 4$  chains, and sampling occurred every 100 generations. The first 7,000 trees were discarded from the final tree as equivalent to the “burnin” phase. In the resulting tree, speciation and duplication events were identified by comparison with accepted species relationships using soft parsimony (4). The divergence order of eutherian mammalian groups was treated as uncertain and reflected in a nonbinary tree. Positive selection was examined in each branch of the phylogenetic tree, using a set of 3 sequential tests described below.

**Analysis of Evolutionary Rates Along Branches of the *Fpr* Phylogenetic Tree.** Positive selection was examined in each branch of the phylogenetic tree using a set of 3 sequential tests. First, a free ratio branches test, using software from the PAML package (5), identified branches with  $d_N/d_S > 0.9$ . These included lineages a ( $d_N/d_S = 999$ ), S2 to D2 ( $d_N/d_S = 1.28$ ), b ( $d_N/d_S = 999$ ), c ( $d_N/d_S = 1.12$ ), d ( $d_N/d_S = 0.91$ ) and e ( $d_N/d_S = 1.18$ ). Six additional lineages also showed  $0.6 < d_N/d_S < 0.9$ , but are not discussed further. For comparison, average  $d_N/d_S$  ratios for all gene duplicate pairs in rat and mouse are extrapolated to be 0.57 and 0.56 immediately following duplication, and decay to 0.06 and 0.07 over time as the genes lose redundancy (6). The average pairwise  $d_N/d_S$  ratio observed between all primate and rodent orthologs is 0.2 (7). Second, individual branch tests (8) indicated statistically significant positive selection in some but not all lineages. (Lineage a:  $d_N/d_S = 999$ ,  $P = 0.086$ , b:  $d_N/d_S = 999$ ,  $P = 0.268$ , c:  $d_N/d_S = 0.91$ ,  $P = 0.0095$ , d:  $d_N/d_S = 1.07$ ,  $P = 0.0045$ , e:  $d_N/d_S = 1.08$ ,  $P = 0.0063$ ). The lineage S2 to D2 did not show evidence of positive selection with this test. Since  $d_N/d_S$  values near 1.0 could result from an averaging of positive and negative selection in different regions of the gene, we used a third SWAPSC test that detects gene regions under positive selection (9). Lineages a, S2 to D2, b, c, and other branches all showed evidence for positive selection ( $P < 0.0001$ ) using SWAPSC. Only branches that were positive with SWAPSC and suggestive from the free ratios and branches tests of PAML are indicated as red on the figure. This is a conservative estimate of positive selection, and it is likely that additional branches underwent positive selection but are not discussed.

Receptor Sequences Used to Generate the Phylogenetic Tree

## Receptor Sequences Used to Generate the Phylogenetic Tree

**Mouse *Fpr1*.** ATGGACACCAACATGTCTCTCCTCATGAACAAGTCTGCAGTGAACCTCATGAATGTATCTGGGAGTACTCAATCAGTATCTGCTGGCTACATCGTTCTGGATGTCTTCTCATATTTGATCTTTGCCGTCACATTTGTCC-TTGGGGTTCTGGGCAACGGGCTCGTGATCTGGGTG-GCTGGTTTCCGCATGAAACACACTGTCACCACCATC-TCTTACTTGAACCTGGCCATTGCTGACTTTTGCTTCA-CTTCCACTTTGCCATTTTACATTGCCAGCATGGTCAT-GGGAGGACATTTGGCCATTTGGTTGGTTTCAATGTGCA-AATTCATATATACTGTAATAGACATAAACCTATTTG-GAAGTGTCTTCTGATTTGCCCTATTGCACTGGACC-GCTGTATTTGTGTTCTGCATCCAGTCTGGGCTCAGA-ACCACCGCACTGTGAGCCTAGCCAAGAAGGTAATCA-TCGTACCCTGGATTTGTGCATTTCTTCTTACATTGCC-AGTTATCATTCGTTTGACCACAGTCCCTAATAGTAG-ACTTTGGACAGGGAAAACAGCCTGTACTTTCCGACTT-CTCCCCTGGACCAAAGATCCTGTAGAGAAGAGGA-AGGTGGCCGTCACCATGCTCAGTGTGAGAGGAATCA-TCAGGTTTCATCATTGGGTTTCAGCACTCCCATGTCCAT-TGTTGCCATTTGCTATGGGTTAATAACCACTAAAAT-TCACAGGCAGGGCCTGATCAAATCCAGCCGTCCTTT-GCGGGTTCTCTCCTTTGTTGTGGCTGCCTTTTCTC-TGCTGGTGGCCATTTCAAGTAGTGGCCCTCATATCC-ACAATCCAAGTCCGTGAACGGTTGAAGAACATGAC-TCCAGGCATTGTAAGTCTTTGAAAATCACAAAGCCC-CTTGGCTTTCTTCAACAGCTGCCTCAATCCAATGCTT-TATGTCTTTATGGCCAGGACTTTCAGAGAAAGACTA-ATCCACTCTTTACCTGCCAGCCTAGAGAGGGCCCTG-ACTGAGGACTCAGCTCAGACCAGTGATACAGGCACC-AATTTGGGGACCAACTCACTTCCCTTTCTGAAAAC-ACTTTAAATGCAATGTAA

**Mouse *Fpr-rs1*.** ATGGAAACCAACTACTCTATCCCTTTGA-ATGGATCAGATGTGGTGTATGATTCTACCATCTC-CAGGGTTCTATGGATCCTCTCAATGGTGGTTGTCTC-CATCACTTTCTTCTTGGTGTGCTAGGAAATGGACT-







ATTTCCATTCTGGGGTGACACTGTTGTAGAGAGGAT-  
 GAACGTGTTTCATTACCATGGCCAAGGTCTCTCTGAT-  
 CCTCCACTTCATTATGGCTTCAGCATACCCATGTCC-  
 ATCATCACAGTCTGCTATGGGATCATCGTTGCCAAA-  
 ATTCACAAAAGCGCATGACTAAATCCAGCCGCTCC-  
 TTACACATCTTCACTGCTGTGGTGGCTTCTTTCTCA-  
 TCTGTTGGTTCCCTTATGAACTAACTGGCATTCTAAT-  
 GGCAGTCTGGCTCAAAGAGATTTTGTTCATGGCA-  
 AATACAAAATCATTATTGTCCTGCTTTACCCAACAA-  
 GTCCTTGGCCTTTTTTAACAGCTGCCTCAACCCAG-  
 TTCTCTACGTTCTCATGGGTCAATACTTCCAAGAAA-  
 GACTGATTGCTCTTGGCCACTAGTTTGGAGAGGG-  
 CCTGACTGAGGTCCCTGACTCAACCCAGACCAGCA-  
 ACACAGACACCAATTCCACTTACCTCCTGAGGAGA-  
 GGGAGTTACAAGCAATGTGA

**Rat *Fpr1*.** ATGAACACCAACATGTCTGCAGTGAACCTCA-  
 TGAATATGTCTGGGAGTACTCGATCAGTATCTGCTG-  
 GCTACATTGTTCTGGATATCTTCTCATATTGATCTT-  
 TGCACCTACCTTTGTGCTTGGAGTCTTGGGCAACGG-  
 GCTCGTATCTGGGTGGCCGTTTCCGCATGAAACG-  
 CACTGTCACCACCATCTTACTTGAACCTTGGCCATT-  
 GCTGACTTTTGTCTCACCTCCACTTTGCCATTTTACA-  
 TCGTCAGCCTGGTCACTGGGAGGAATATGGCCATTG-  
 TTGGTTTCACTGTGCAAGTTCATATATACTGTAATAG-  
 ACATAAACCTATTTGGAAGTGTCTTTCTGATTGCC-  
 TCATAGCATTGGATCGCTGTGTTTGTGTCCTGCATCC-  
 AGTCTGGGCTCAGAACCACCGCACCGTGACCCTGGC-  
 CAAGAAGGTAATCATTGTACCCTGGATTTGTGCATT-  
 TCTCCTTGTGGCAGTTATCATCTGTTGACACACA-  
 TCCCTAATCGACTTGGACCAGGAAAACAGCCTGTG-  
 GCTTTAGACTTCTCACCTGGACCAAAGATCGTGCA-  
 GAAAAGGATAAAGTAGCTATCACCATGTATACTGTC-  
 AGAGGAATAATCAGGTTTATCCTTGGGTTGAGCACT-  
 CCCATGTCCATTGTAGCTATTTGTTATGGGTTAATA-  
 GCCACTAAAATCCACAGGCAAGGCTTATCAAATCC-  
 AGCCCTCCCTGCGAGTCTCTCTTTTGTGTGGCTG-  
 CCTTTTTCTCTGTGCTGGTGGCCATTTCAAGTATGG-  
 GCCTCATACGCACAATCCAAATCCGTGAACACTTGA-  
 GGAACATACTCAAAGCACTCTAACTGCTATGAAAA-  
 TCACAAGCTCTTTGGCTTTCTTCAATAGCTGCCTCA-  
 ACCCAATCCTTTATGTCTTCATGGGCCAGGACTTCA-  
 GACAAAGACTAATCCACTCCCTACCTGCCAGTCTAG-  
 AGAGGCCCTGAGTGAGGACTCAGTCTCAAACCAAGT-  
 GACACAGGCACCAACTTGGGAGCCAACCTCTATTCT-  
 GAAAACCCAGCAAATGCAATGTAA

**Rat *Fpr-rs2a*.** ATGGAAGCCAACACTATTCCATCCCTCTGAA-  
 TGTATCAGAAGTGGTGTCTATGATTCTACCATCTCC-  
 AGAGTTTGTGGATCCTCACAATGGTGGTTCTCTCC-  
 ATCACCTTTGTCTGGGTGTGCTGGGTAATGGACTA-  
 GTGATCTGGGTAGCTGGATTCCGGATGGTACACACT-  
 GTCACCCTACCTGTTTTCTGAATCTAGCTTTGGCTG-  
 ACTTCTCTTTCACAGTACTCTACCATTCTTTGTCTAT-  
 CTCAATTGCTATGAAAGAAAATGGCCTTTTGGAT-  
 GGTTCCTGTGTAATAGTTTACATTTGTAGTAGACA-  
 TAAACCTCTTGGAAAGTGTCTTCTGATTGCTTTAA-  
 TTGCCTTGGACCGCTGCATTTGTGTCCTGCATCCAGT-  
 CTGGGCTCAGAACCACCGCACTGTGAGCCTGGCTAG-  
 GAAGGTGGTTGTTGGGCCCTGGATTTTAGCTCTGAT-  
 TCTCACTTTGCCATTTTTATTTTTCATGACTACAGTT-  
 AGAATTCTCGAGGTAATGTGTACTGTACATTCAAC-  
 TTCGCATCTGGGGTAACTGCTGTAAGAAGAACTATTG-  
 AACATAGCTAACACTTTTGTAAACAGTTAGAGGGAG-  
 CATCAGGTTTCAATTATTGGCTTCATAATGCCTATGTCC-  
 ATTGTTGCCATCTGCTATGGACTCATCGCTGTCAAG-  
 ATCCACAGAAGAGCACTTGTAAATCCAGCCGTCCA-  
 TTAAGAGTTCCTTACAGCAGTTGTGGCTTCCTTCTTT

ATCTGTTGGTTTCCCTTTCAACTGGTGGCCCTTTTA-  
 GGTACAATCTGGTTTAAAGAGTCAATTGTTTAGTGGT-  
 CGTTACAAAATTCTTGACATGTGGGTTACCCCAACC-  
 AGCTCATTGGCCTACTTCAATAGTTGCCTCAATCCA-  
 ATGCTCTATGTTTTTTCATGGGCCAGGACTCCGAGAG-  
 AGACTGATTTCATTCCCTGCCTTCCAGTCTGGAGAGA-  
 GCCCTGAGTGAGGACTCTGGCCAAACCAGTGATACA-  
 GGCATCAGTTCTGCTTTACCTCCTGTAAACATTGAT-  
 ATAAAAGCAATATGA

**Rat *Fpr-rs2b*.** ATGGAAGCCAACACTATTCCGTCCTCTGAA-  
 TGTATCAGAAGTGGTGGTCTATGATTCTACCATCTCC-  
 AGAGTTCTGTGGATCCTCTCAATGGTGGTTCTCTCC-  
 ATTACATTTGTCTTGGTGTGCTGGGTAATGGACTA-  
 GTGATCTGGGTAGCTGGATTCCGGATGGTACACACT-  
 GTTACCCTACTTGTATCTGAATTTAGCTTTGGCT-  
 GACTTCTCTTTCACAGCAACTCTACCATTCTTTGTCA-  
 TCTCAACTGCTATGAAAGAAAATGGCCTTTTGGCT-  
 GTTTTCTGTGTAATTAGTTTACATTGTGGTAGACA-  
 TAAACCTCTTTGGAAGTGTCTTCTGATTGCTTTAA-  
 TTGCCTTGGACCGCTGCATTTGTGTCCTGCATCCAGT-  
 CTGGGCTCAGAACCACCGCACTGTGAGCCTGGCTCG-  
 GAAGGTGGTTGTTGGGCCCTGGATTTTAGCTCTGAT-  
 TCTCACTTTGCCATTTTGTGTTTTCATGACTACAGTT-  
 AGAGTTCTGGAGGTAATGTGACTGTACTTCAAC-  
 TATGCATCCTGGGGAAAAACTGTTGAAGAAGTGTCT-  
 GGACGTAGCAAACACTTGTGAAACAGTTAGAGGGGA-  
 CCATCAGGTTTCGTTATTGGCTTCACAATGCCCATGT-  
 CCATTGTGGCCATCTGCTATGGACTCATCGCTGTCA-  
 AGATCCACAGAAGAGCACTGTTAATCCAGTCCGTC-  
 CCTTACAGTCTTACAGCAGTTGTGGTTTCTTTCTT-  
 TATCTGTTGGTTTCCCTTTCAACTGGTGGCCCTTTTA-  
 GGTACAATCTGGTTCAAAGAGCTATTGTTTAAACT-  
 CGTTACGGAATTCTTAAGATGTGGGTTAACCCAAACC-  
 AGCTCATTGGCCTACTTCAATAGTTGCCTCAATCCA-  
 ATGCTCTATGTTTTTTCATGGGCCAGGACTCCGAGAG-  
 AGACTGATTTCATTCTGCTTCCCTTCCAGTTTGGAGAGA-  
 GCTCTGAGTGAGGACTGTGGCCAAACCAGTGATACA-  
 GGCACAAATTCTGCTTTACCTCCTGCAGACATTGAG-  
 ATAAAAGCAATATGA

**Rat *Fpr-rs2c*.** ATGGAACCAACTACTCCATTCCAATGAG-  
 TGATCAGAAGTAATGGTAAATGATTCTACCATCTC-  
 CAGAGTTCTGTGGATCCTCACAATGGTGGTTCTGTC-  
 CATCACATTTGTCTTGGTGTCTGGGTAATGGACT-  
 AGTGATCTGGGTAGCTGGATTCCGGATGGCACACAC-  
 TGTACCACCACCTGTTATCTGAATCTAGCTTTGGCT-  
 GACTTCTTTTTTCACAGCACTCTACCATTTTTTCATCA-  
 TCTCAACTGCTATGGAAGAAAATGGCCTTTTGGCT-  
 GTTCTCTGTCAAATTAATTTATATTGACAGATG-  
 TCAATGGAATTGGAAGTATCTTCTGATTACTTTAA-  
 TTGCCTTGGACCGCTGTATTTGTGTCGTGCATCCAG-  
 TCTGGGCTCAGAACCACCGCACTGTGAGCCTGGCTA-  
 GGAAGGTGGTTATTGGACCTGGATTTTGGTCTGA-  
 TTCTCAGTTTGCACATTTTTATTTTTCATAAGTACATA-  
 TAGAGTTTCTGGAGGGATGTGACTGTGTATACTA-  
 CTTTCCACTGGGGTAACTGACTGATGAGGAAATGTT-  
 GAACACAGTTTTTCAATTTTACAACCTGCTCTAGGGAT-  
 CATCAAGTTCATTATTATCTTTCATAATACCCATGTCC-  
 ATTGTTTCCATCTGCTATGGACTCATTGCTGTCAAG-  
 ATCCAGAGAAGAGCCCTGTTAATTTAGCCGTGCT-  
 TCACAGATCCTAAAGCCAGTGTGGTCTCTCTTTT-  
 ATCTGTTGGTTTCCCTTTCAACTGGTGGCCCTTTTAA-  
 GCCTAGTGTGGTTTAAAGAGAGATTGTTAAATGAT-  
 GAGTATAAAATTATTGACATGTTGATTATGCCAAC-  
 AACTCATTGGCCTTCTTCAATAGTTGCCTCAATCCA-  
 TACTCTATGTTTTCTTTGGCCAGGACTTCCGAAAA-  
 AGACTGATTTCATTCTGCTTCCAGTTTGGAGAGA-





AAACCAAGTGGACATGAACATCTTACTGATCTGGCTGGACGCCCTGGCCCTCCTTCTGATCTGTGCCACAGCTGCCTCAACCCCTGCTCTACGCCTTCTGAGCCCGACTTTCGGAAACAACACTGTTCCGCTGCCTGCCGGCCATTCTGGACTGTGGTGGCCTTGGGAGGAGTCCGAATCTGCCCATCTGTGGGGAACAATTGGACCAAGCCGACCCGAGACTGAGTGCCAGAGCTGTGA

**Platypus 4.** ATGGCATCAGATTCACCCCTGAGACTCCCA-GCCACCAGCCCACCATCTGGGGAGGACAAGCCCTCC-GACTTCCCTGTTGACAGCCAGCCACCATCAACATC-TTCACCGTCTCTTACATCTTACCTTCTTTCGCA-GCGTGTGTGGCAACGGGTTGGTACATCTGGGTGGC-GGCTTCCGCTGCCCTCACGGTCAACCGCATCTGG-TACCTCAACCTGGCCGTGGCCGATTTGGCCTTACC-TTGTCCCTGCCGTTTCTGGCGGTGGTTTTGGCCCTG-AGCCACCAGTGGGTACCTGCAAGGTCATCGCGGTC-GTGCTGGTGTCAACCTTTCGCCAGCATCTTCCAG-CTGAGTCTCATCTCTGTGGACCGTGCCTGTGCGTG-TACTGGCCCTGTGGCCCGCCGACACCGCACCCCT-TGGCGGGCAGGGCTGGGGGTTGGGGCCGTCTGGCT-TCTGGCCCTGGCCTGCGTGGTCCCGAACGTGCTGTT-CTTGACTGGTTGGCCATCCAGGGCTCGGCCAACGC-CAGATGCTACAAAATCTTTGACTCCTGGAACCAGAC-CCGTGGCGATGGGGAGCGGGGCAGCAATTGGCCA-GGAGTCAAGCTTTGGCCTTCCGGGCTGGCCACTTCA-CTTACACTTCTGCTGCCCTGGCCCTCATTTGGGG-CTGCTCCGGCCTCATCGCCTTCAAGCTGCGGAGGAA-GCAGGAGCAGCCCAGTAGGCCCTTCCGCGTGCTGGC-CTCCGTGGTGGCCGCTTCTTCGTCTGCTGGTTCCCC-TCCACCTCCTGGGGCTGCTGCAGCTGGGGTCGGGG-GACCCTGTGAGCTCGGAGCTGAGGTCCCACTTTC-ACCCTTCTGGGGCCGCTGACCTTGTGCTTATGCTGC-CTCAACAGTCTGCTTGAACCCCTTCTCTAGCTTTCC-TGGGCCGGGACTTCCGGGACCATCTGATCCGCTCCC-TGCCACCGCCCTGGAGCGGGCCCTCAGCGAGGAGC-CTGACACAGCCCAAAGCAGTCAATCAGTAGAAT

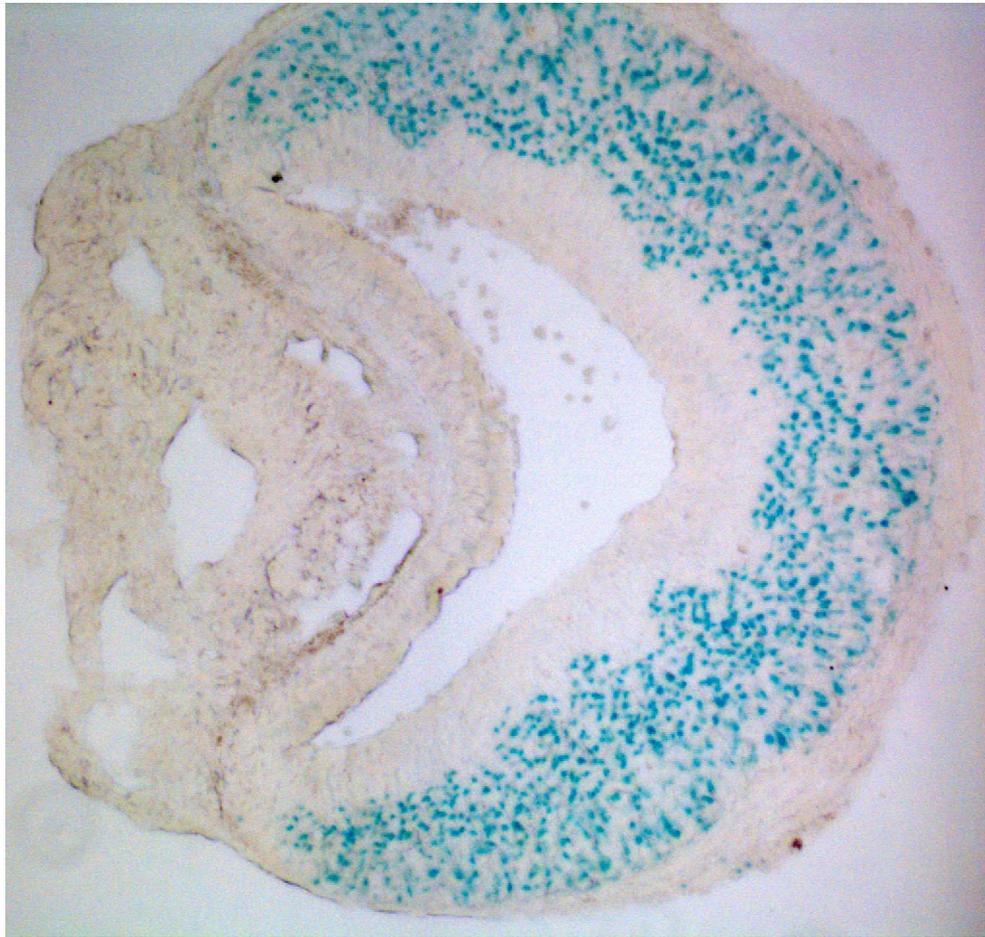
**Platypus 5.** ATGGAGAGCGGGTCGCCGTTCCCGCGCTG-CCTCCGACGGCCCCGCGCCCCGCCGCTACGCC-TCGGCCTCCTCAGACAGGACGCGGGATGCCCTCCAC-GTCTTCTACGCGGTACATCTTCTCCGCCACCTTCCCTC-TGGGCACGGCCGCAACGGGCTGGTACATCTGGGTG-TCCGGCTTCCGCATGACCCGAACGGTGAACGGCGTC-TGGTACCTCCACCTGGCCGTGGCCGACTTCGCCTTCT-GCCTTCTCCTGCCCTCGCCGCGCCAGGCGCCCTGGGCTTCCACTGGCCCTTCGGCCGCGCTGTCA-AAGTCTACAACACTCGTTGGGCGCCCTCAACCTTTCG-CCAGCGTCTTCTGCTGACCCCTCATCTCCGCCGACCG-CTGCGTCTGCGTCTGCGCCCCGTCTGGGCCAGCG-CCGCCGTTCCCGGGGCGGGCCGCCCTGGCCGGCGT-GGGGGCCTGGCTCCTGGCCCTGGCCTTGAGCGTGCC-CCAGTTCTTATTACAGGACACCGACGGGGAGGGCG-AGCAGTCCACTGCTACAACAACCTTCGACCCCTGGA-ACGAGACCCGGGGGACGAGGAAGTGGGAGGGGA-ACTGGAGAAGACCCGGATGGTGGCCGTGGCGGTCA-CCCGCTGGTCTGGGCTTCGTCTGCCGCTGGTAC-CATCGGGGCTGCTACGCCCTGGTCTCGTCCAGCT-GGCGAGGAAGGGCGAGCTGCGGGGGACCGGCCGG-CCGCTGCGGGTGTCTGCGCCGTGGTGTGGCTTTT-TTCTCTGTGGCTTCCCGTCCACTGGCGTGGCCTCC-TGACGTGGTGGCCTACGTGCGCGGGTCCAGGCC-TGTTCCGCCGCGGTGGGGGTCGTGGGGCCGCTGGGC-GTCTGCCTGATTTCTGGCGTACAACAGCTTAGCCTCA-ATCCCGAGGCATTAATCCTTTCATGGACCGGACTTT-CAGGGGAAGCATTTGTTTTCTAACTACCCTGACCCG-CCATCCATGTAG

**Platypus 6.** ATGGAGAACGTCACGTCGAGTCCGCTCACT-TCTAAGACCCTCACGGCCACAGAGGAACCCCTCGCC-GAGGACTCGGACGCCATCACCTTCCCCCTCCAGGTC-TTCAGCATGATCATCTACTCTCGGCCTTCTTGTAG-GCACGGTGGCAACGGGCTGGTACATCTGGGTGGCC-GGCTTCCGCATGACCCGACGGTACCACCATTTG-TTCTCAACCTGGCCGTAGCCGACTTCATCTTCTGCC-TCTTCTGCCATCAGTGTGACCCAAGTGGCCCTGG-ACTTCCACTGGCCCTTCGGCTGGCTGGGCTGCAAAT-TCTTACCACCCTTGGCGTCTTCAACCTTTCGCCAG-CGTCTTCTGCTGACCTCATCTCCATTGACCGCTGC-GTCTCCATCTCGGGCCCATCTGGGCCGGAACCA-CGCACCCCGAGGCGGGCGGCCCTGGGGGCCGGGGG-CATCTGGCTCCTGGCCCTGGTCTCTGCATCCCGACG-CTGCTTACAGGGACACAGAGATGTATGAAAATGG-CTACGTGTCTGCTTTTTAAATTTTCGACCCCTGGAA-TGAGACCCCGAGGACCCCGAGCTTTCACGGGTGAC-GGAGGCCAGAGGTACAAGGCCAAGTGGCCACTC-GCTTCGTGTTCGGCTTCGCGATCCCTCTGGTCTGTCAT-CTGCAGCTGCTACGGCCTGATCGCGGCCCGGCTCCA-GGACAGGCAGGCGATGCGCTCCAGACGACCCTTCC-GCGTGTGGCCCGCGTGGTGGCCGCTTCTTCTTCT-GCTGGTTGCCCTTCCACATCGTGTCTTTCATGGAGCT-CGTGTTTACTCTGAACAGTTGGAGGACCTGGGCAC-CTGGGTGATACTCTGGGTTCTCTCAGCAGTCTG-TGCCTACGTCAACAGCTGCCTGAACCCCATTTTAC-GTCTTTCATGGGCCGGGACTTCCGAGAGCGGCTGCTC-CGGTCCCTGCCCGCTCCCTGGAGCGCGCCCTCGCG-GAAGAGTCGGCCGACTCTGACTTCAAGGGGAGCAA-CTTGTGTCCACTCCCCAGGACACCGAGTCCAGGA-GGTGTGA

**Opossum 1.** ATGGAGAACTCCTTGGAGTCTCTTCCAAAT-GCTTCAGACACCCTTACCCTGTGGAACGTTTACCA-TCTGCCATCCACCAAACCTTCTGGATCATTGGTCTCA-TCATCTACTGCTTAACCTTTGTGTGCGGCATAACT-GCAATGGGCTGGTGTACTGGGTGACAGGCTTCCGG-ATGACTCGCACAGTACCACCTGTCTTGTTCCTCAACT-TGGCCTCAGCTGACTTCACCTTCACTGCCTTCTGCC-CTTCATCATCATTAATACCATCCTGCAACCCATTGG-CCCTTTGGTTGGTTTCTCTGCAAACCTATTAGCTCCC-TGTCTGTCTTTAATATGTCTGCCAGTGTCTTCTGCT-GACACTCATCTCTTTGACCGCTGTGTCTCTGTCTC-TGGCCAGTGTGGGCCAGGAATCACCGCACACCCGCT-CGGGCAGCCATGGCTGCTGTAGGGGCTGGATTTTT-GCCCTGGCTATCTCTTTCCCAACAGTCACTTCAAGA-CTACAGTACAGAGAATGGGAAAACCTTTTGCTACT-CTGACTTGGACCCCTGGAATGAGACAGGAGCTGAT-GAAGCCCTGTATGATGCCCTGGCTGAGAGCCGCTC-TGGTCCCTGTGTGCTCAGCCGATTCTTCTTGGTTTTG-TCATCCCTCTAGTCATCATCAGTGTCTGCTATGCCCT-TATAGCAGCCAGGCTCTGCAGTGGAAATGAAGATGG-CCAAGTCCAGTAGGCCCTTCAAGGTCTCACAGCTG-TGGTGAAGTGCCTTCTTCTCTGTGGCTCCCTCATCA-TGTGATGTCTATGATAACGGTCTCTGCGTACCAACA-GCCACACCTGAAACATCTTTTGCCTTCCCTCAGTCTC-TCTCCTCCTCCCTGGTATTTCTCAACAGTTGCCTCA-ACCCTTACTCTATGTCTTTCATAGGTAGGGACTTCAG-GGAGAGACTCCTCAGAACCCTGCCAGCTGCCCTAGA-GCGGGCTCTCAGTGAGGAATCTGGCCCAACGGGCA-TCACAGGGAAGTCTCAACTCTGCCCTGCAGCCA-CTGATGTGGAAGCCAGGGGTTATGA

**Opossum 2.** ATGGAGAACTCCTTGGGGTCTCTTCCAAAT-GCTTCAGACATCCTTCTCCCTGAGGAGCCTTACCAT-CTATCATTACCAAACCTTCTGGACCATCGCTCTCAT-CATCTACTGCTTAGCCTTTGTGCTGGGCATAACTGG-CAATGGGCTGGTGTACTGGGTGACAGGCTTCCGGAT-





**Fig. S1.** Endogenous  $\beta$ -galactosidase activity of mouse VNO sensory neurons. A 14  $\mu\text{m}$  coronal section of a C57BL6/J mouse VNO was treated with glutaraldehyde fixative and stained overnight with the  $\beta$ -galactosidase substrate X-gal (1). Most or all sensory neurons were labeled (blue cells), whereas other cells in the VNO were not.

**Table S1. Numbers of VNO neurons labeled by *Fpr*, *V1r*, and *V2r* probes**

Probe	No. genes*	No. cells	No. sections	Average no. cells per section	No. animals (male/female)
<i>Fpr-rs1</i>	1	182	47	3.9	2/3
<i>Fpr-rs3</i>	1	514	91	5.6	4/3
<i>Fpr-rs4</i>	1	288	65	4.4	4/3
<i>Fpr-rs6/rs7</i>	2	405	87	4.7	4/4
<i>V1ra2/11</i>	6	238	18	13.2	1/1
<i>V1ra9</i>	1	26	20	1.3	1/1
<i>V1rf5</i>	1	547	79	6.9	3/3
<i>V1rg8</i>	1	32	20	1.6	1/1
<i>V1rj2</i>	3	1371	80	17.1	3/3
<i>V2ra</i>	17	2184	19	115.0	1/1

\*, number of genes encoding RNAs predicted to hybridize to each probe based on sequence relationships.

**Table S2. Numbers of VNO neurons colabeled by different probes**

Probe A	Probe B	A <sup>+</sup> cells	B <sup>+</sup> cells	A <sup>+</sup> B <sup>+</sup> cells	A <sup>+</sup> B <sup>+</sup> /A <sup>+</sup> (%)	A <sup>+</sup> B <sup>+</sup> /B <sup>+</sup> (%)	No. animals (male/female)
<i>Fpr-rs1</i>	<i>Fpr-rs3</i>	230	377	0	0	0	4/3
<i>Fpr-rs1</i>	<i>Fpr-rs4</i>	148	286	1	0.7	0.3	3/3
<i>Fpr-rs1</i>	<i>Fpr-rs6</i>	140	335	0	0	0	3/3
<i>Fpr-rs3</i>	<i>Fpr-rs6</i>	160	116	0	0	0	1/3
<i>Fpr-rs4</i>	<i>Fpr-rs3</i>	172	286	1	0.6	0.3	3/6
<i>Fpr-rs4</i>	<i>Fpr-rs6</i>	166	202	1	0.6	0.5	3/3
<i>Fpr-rs3</i>	<i>Fpr-rs3</i>	187	159	157	84.0	98.7	2/5
<i>V1rd11</i>	<i>V1ra2</i>	149	128	0	0	0	0/1
<i>Fpr-rs1</i>	<i>Gα<sub>12</sub></i>	141		5	3.5		4/3
<i>Fpr-rs3</i>	<i>Gα<sub>12</sub></i>	250		237	94.8		3/3
<i>Fpr-rs4</i>	<i>Gα<sub>12</sub></i>	133		131	98.5		3/3
<i>Fpr-rs6</i>	<i>Gα<sub>12</sub></i>	89		87	97.8		4/1
<i>V1ra2</i>	<i>Gα<sub>12</sub></i>	229		224	97.8		3/3
<i>Fpr-rs1</i>	<i>Gα<sub>o</sub></i>	140		131	93.6		3/3
<i>Fpr-rs3</i>	<i>Gα<sub>o</sub></i>	163		7	4.3		3/4
<i>Fpr-rs4</i>	<i>Gα<sub>o</sub></i>	147		3	2.0		3/3
<i>Fpr-rs6</i>	<i>Gα<sub>o</sub></i>	62		3	4.8		3/2
<i>V1ra2</i>	<i>Gα<sub>o</sub></i>	187		9	4.8		3/3
<i>Fpr-rs3,4,6 mix</i>	<i>V1ra2, V1rc33</i>	286		1	0.3		3/3
<i>Fpr-rs3,4,6 mix</i>	<i>V1rb4, V1rd8</i>	216		2	0.9		3/3
<i>Fpr-rs3,4,6 mix</i>	<i>V1re11, V1ri10</i>	637		1	0.2		3/3
<i>Fpr-rs3,4,6 mix</i>	<i>V1rf5</i>	418		0	0		3/3
<i>Fpr-rs3,4,6 mix</i>	<i>V1rg8, V1rh20</i>	296		2	0.7		3/3
<i>Fpr-rs3,4,6 mix</i>	<i>V1rj2</i>	228		0	0		3/3
<i>Fpr-rs1</i>	<i>V2ra</i>	106		0	0		3/2

Dual fluorescence in situ hybridization was used to compare VNO neurons labeled by different probes or probe mixes (probe A versus B). The *V1r* probes are predicted to hybridize to RNAs encoded by the following numbers of *V1r* genes, as determined by ClustalW analysis of intact *V1r* genes (J. Young and B. Trask, unpublished data): *V1ra2* (6 genes), *V1rb4* (8 genes), *V1rc33* (4 genes), *V1rd8* (12 genes), *V1rd11* (12 genes), *V1re11* (1 gene), *V1rf5* (1 gene), *V1rg8* (1 gene), *V1rh20* (2 genes), *V1ri10* (5 genes), and *V1rj2* (3 genes). The *V2ra* probe is predicted to hybridize to RNAs encoded by 17 *V2r* genes, as determined by megaBLAST (NCBI) analysis against intact *V2r* genes.